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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court West, Suite B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
ADCC Bioassay Effector Cell (Mouse)
Catalog #: 79733

Background
Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune defense mechanism involving an effector cell lysing a target cell on which antibodies have bound to specific antigens on the target cell membrane.

The typical ADCC involves activation of natural killer (NK) cells by antibodies. NK cells express Fc receptors, mostly FcγRIV in mouse, on its cell surface. These Fc receptors recognize and bind to the Fc portion of an antibody, usually IgG2a, IgG2b and IgG3 in mouse, which has bound to the surface of a pathogen-infected target cell. Once the Fc receptor binds to the Fc region of IgG, the Natural Killer cell releases cytokines such as IFN-γ and cytotoxic molecules that attack the pathogen-infected target cell.

Description
The mouse ADCC Reporter cells (mFcγRIV /NFAT-Jurkat cells) are recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of mouse FcγRIV (NM_144559.2).

Applications
Characterize the Fc effector function of antibodies and measure ADCC activity in cellular assays.

Format
Two vials containing ~ 2 x 10^6 cells in 1 ml of 10% DMSO in FBS.

Storage
Store in liquid nitrogen immediately upon receipt. Do not store for long-term at -80°C or on dry ice.

Mycoplasma Testing
This cell line has been screened using the Venor™ GeM Mycoplasma Detection Kit, PCR Based (Sigma, #MP0025) to confirm the absence of Mycoplasma contamination.

Culture Medium:
Thaw Medium 2 (BPS Cat. #60184): RPMI1640 medium (Life Technologies, #A10491-01) supplemented with 10% FBS (Life Technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 2G (BPS Bioscience, #79734): Thaw Medium 2, 0.5 mg/ml G418 (Life Technologies #11811031) and 2.5 µg/ml Puromycin (InvivoGen #ant-pr).

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Recommended Culture Condition:

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin or Puromycin)**, spin down cells at 1000 rpm, and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 **(no Geneticin or Puromycin)**.Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 2 **(no Geneticin or Puromycin)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 2G **(contains Geneticin and Puromycin)**.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1 x 10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with higher ratio.

To freeze down the cells, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.

It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Functional Validation and Assay Performance

The functionality of the cell line was validated using an ADCC reporter assay. In this ADCC assay, the engineered Jurkat cells stably expressing mouse FcγRIV, and firefly luciferase gene under the control of NFAT response elements (mFcγRIV /NFAT-Jurkat cells) are used as effector cells. When the Fc effector portion of antibodies that bind to target antigens on the target cell surface also binds to mFcγRIV on the cell surface of effector cells, cross-linking of the effectors and target cells occurs, leading to the activation of NFAT pathway in the effector cells, which is the early step in ADCC MOA pathway. Therefore, the NFAT responsive luciferase reporter is induced.

The following assays are designed for a 96-well plate format. To perform assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

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Materials Required but Not Supplied

- **Assay Medium 2A (BPS Bioscience, #79621):** RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% low IgG FBS (Thermo Fisher, #16250078), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).
- Anti-CD20 mouse IgG2a (InvivoGen #hcd20-mab10)
- Human B cell WIL2-S (ATCC #CRL-8885)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

Analysis of mouse ADCC - Jurkat reporter activity in response to mouse anti-CD20 IgG2a antibody in co-culture with WIL2-S.

1. One or two days before assay, seed the mouse ADCC cells in Assay Medium 2A (#79621) and grow for 1 or 2 days.

2. On the day of assay, seed WIL2-S cells in a white opaque 96-well plate, at 1 x 10^4 cells/well in Assay Medium 2A. Add anti-CD20 mouse antibody IgG2a (InvivoGen Cat# hcd20-mab10) or test antibody or control antibody, mix well and incubate for one hour at 37°C with 5% CO_2.

3. Harvest the mADCC/NFAT-reporter-Jurkat cells by centrifugation and resuspend in Assay Medium 2A. Add 6 x 10^4 cells/well to the WIL2-S cells incubated with either anti-CD20 or nonspecific negative control antibody. Set up each treatment in at least triplicate.

4. Add 100 µl of Assay Medium 2A to cell-free control wells (for determining background luminescence). Incubate the plates at 37°C in a CO_2 incubator for 5 hours.

5. After ~5 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system (#60690) following the provided protocol. Add 100 µl of ONE-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Note: Assay conditions have been optimized for anti-CD20 IgG2a and WIL2-S co-culture. When testing with other antibodies or target cells, different assay conditions may be required for optimum results, such as assay time, cell numbers, and target : effector cells ratio (6:1 in this assay protocol).

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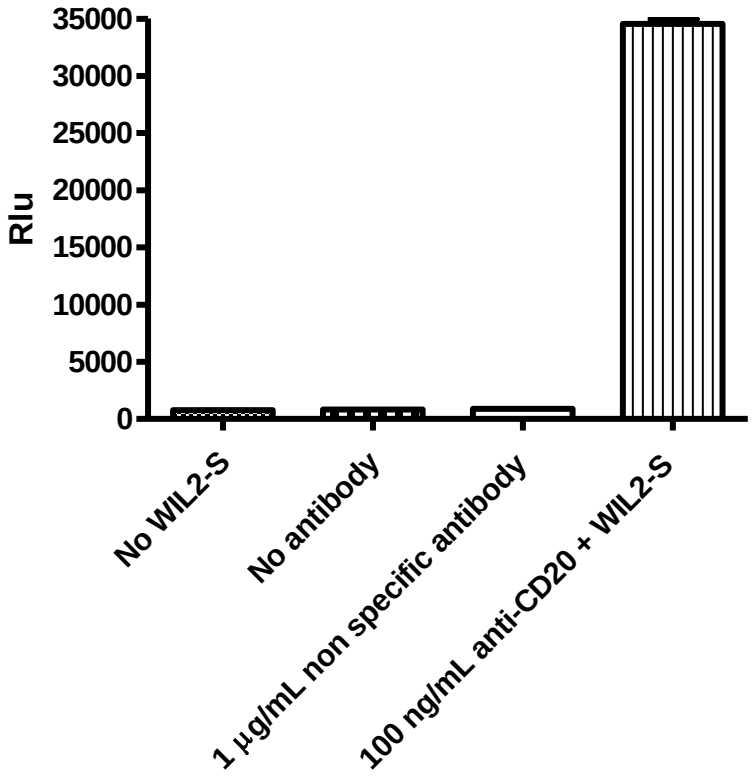
Figure 1. ADCC response to anti-CD20 mouse IgG2a antibody, in mADCC Effector Cell (FcγRIV /NFAT-Jurkat cells).
Anti-CD20 (InvivoGen #hcd20-mab10), nonspecific control antibody, or assay medium (no antibody) was incubated with ADCC Bioassay Effector Cell (mFcγRIB /NFAT-Jurkat cells), with or without target cells (human B cell WIL2-S). After ~5 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Bioscience, #60690) was added to the cells to measure NFAT activity.

A. Specificity of the ADCC response to anti-CD20. Anti-CD20 induced NFAT luciferase reporter activity in mADCC Bioassay Effector Cell (FcγRIV /NFAT-Jurkat cells) co-cultured with WIL2-S.



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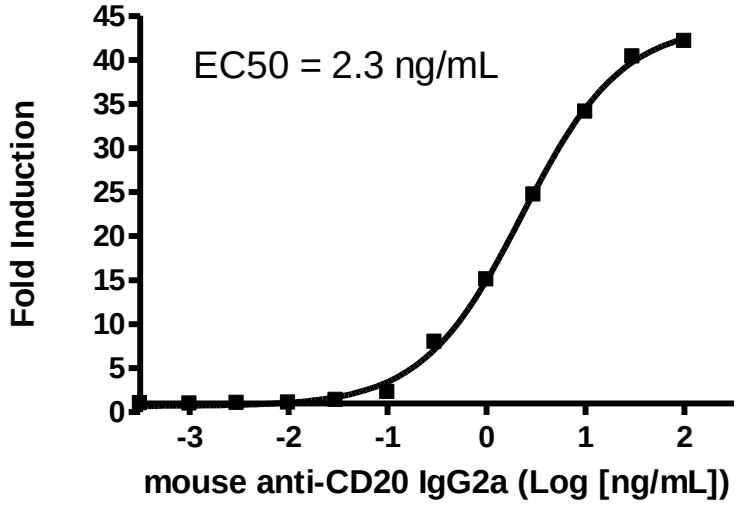
B. Dose response of mouse anti-CD20 IgG2a in mADCC Bioassay Effector Cell (mFcγRIV /NFAT-Jurkat cell. The result is shown as fold induction of NFAT luciferase reporter. EC50 = 2.3 ng/ml



References

Bhavin S. Parekh, *et al.* 2012. "Development and validation of an antibody-dependent cell-mediated cytotoxicity-reporter gene assay." mAbs, **4(3):**310-318, doi:10.4161/mabs.19873

Carlos Rosales. 2017. "Fcγ Receptor Heterogeneity in Leukocyte Functional Responses." Frontiers in Immunology, **8:** 280, doi:10.3389/fimmu.2017.00280

Related Products

Product Name	Catalog #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 mL
ONE-Step™ Luciferase Assay System	60690-2	100 mL
ONE-Step™ Luciferase Assay System	60690-3	1 L
Thaw Medium 2	60184-1	100 mL
Thaw Medium 2	60184-2	500 mL
Growth Medium 2G	79734	500 mL
Assay Medium 2A	79621-1	100 mL
Assay Medium 2A	79621-2	500 mL
ADCC Bioassay Effector Cell, F variant (Low Affinity)	60540	2 vials
ADCC Bioassay Effector Cell, V variant (High Affinity)	60541	2 vials
ADCP Bioassay Effector Cell FcγRIIa (H variant)	71273	2 vials